

Bulletin No. 34 from the Department of Surgery,

University of Lund, S-221 85 LUND,

1982

ENTERO-PANCREATIC INTERACTIONS

Effects of duct-occlusion, dietary fiber and
pancreatic enzyme substitution on the exocrine
and endocrine pancreas



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ENTERO-PANCREATIC INTERACTIONS.
EFFECTS OF DUCT-OCCLUSION, DIETARY FIBER AND
PANCREATIC ENZYME SUBSTITUTION ON THE EXOCRINE
AND ENDOCRINE PANCREAS

by
Gunnar Isaksson

Lund 1982

Better is a dinner of herbs where love is,
than a stalled ox and hatred therewith.
(Proverbs 15:17)



To Gunvor
Karin Britta Erik

The present thesis is based on the following papers, which in the text will be referred to by their Roman numerals:

- I. G Isaksson, I Ihse, I Lundquist. Influence of pancreatic duct ligation on endocrine and exocrine rat pancreas. Accepted for publication in *Acta Physiol Scand*.
- II. G Isaksson, I Lundquist, I Ihse. Effects on the exocrine and endocrine pancreas of duct occlusion with two different tissue glues in the rat. Accepted for publication in *Eur Surg Res*.
- III. G Isaksson, I Lundquist, I Ihse. In vitro inhibition of pancreatic enzyme activities by dietary fiber. *Digestion* 24:54-59, 1982.
- IV. G Isaksson, I Lundquist, I Ihse. Effect of dietary fiber on pancreatic enzyme activity in vitro. The importance of viscosity, pH, ionic strength, adsorption, and time of incubation. *Gastroenterology* 82:918-924, 1982.
- V. G Isaksson, P Lilja, I Lundquist, I Ihse. Influence of dietary fiber on exocrine pancreatic function in the rat. Submitted.
- VI. G Isaksson, N-G Asp, I Ihse. Effects of dietary fiber on pancreatic enzyme activities of ileostomy evacuates and on excretion of fat and nitrogen in the rat. Accepted for publication in *Scand J Gastroenterol*.
- VII. G Isaksson, I Lundquist, B Åkesson, I Ihse. Effects of dietary fiber on intraluminal pancreatic enzyme activities and on fat absorption as examined with the triolein breath test in patients with pancreatic insufficiency. Submitted.
- VIII. G Isaksson, B Åhrén, I Ihse, I Lundquist. Effects of pectin on glycaemia and insulinaemia after starch loading in normal, alloxan diabetic, and pancreatic duct-occluded rats. Submitted.
- IX. G Isaksson, I Ihse. Pain reduction by an oral pancreatic enzyme preparation in chronic pancreatitis. Accepted for publication in *Dig Dis Sci*.

CONTENTS

1.	PURPOSE AND PLAN OF THE PRESENT INVESTIGATION	6
2.	GENERAL BACKGROUND	7
2.1.	Pancreatic function	7
2.2.	Entero-acinar interactions	7
2.3.	Entero-insular interactions	9
2.4.	Importance of the intestinal activities of pancreatic enzymes for exocrine and endocrine pancreatic function	9
2.5.	Treatment in chronic pancreatitis	10
3.	ANIMALS, PATIENTS AND MATERIAL	11
3.1.	Rats	11
3.2.	Patients and healthy volunteers	11
3.3.	Chemicals, drugs and digestive media	11
4.	METHODS AND EXPERIMENTAL DESIGN	13
4.1.	Biochemical assays	13
4.2.	In vitro experiments	13
4.3.	Experiments in the rat	14
4.3.1.	Pancreatic duct occlusion	14
4.3.2.	Studies of secretagogue and trophic effects of dietary fiber, and oral trypsin inhibitor	15
4.3.3.	Fat and protein absorption experiments	15
4.3.4.	Starch loading experiments	15
4.4.	Human studies	16
4.4.1.	Lundh test	16
4.4.2.	Triolein breath test	16
4.4.3.	Effects of enzyme substitution on pain	16
4.5.	Calculations	17
5.	EFFECTS OF PANCREATIC DUCT OCCLUSION ON PANCREATIC FUNCTION	18
5.1.	Background	18
5.2.	Occlusion of the pancreatic duct system in the rat	18
5.2.1.	Exocrine effects	18
5.2.2.	Endocrine effects	19
5.3.	Exocrine-endocrine relationships	20
5.4.	Summary	22
6.	EFFECTS OF DIETARY FIBER ON PANCREATIC ENZYME ACTIVITY IN VITRO	23
6.1.	Background	23
6.2.	Fiber preparations	24
6.3.	Fiber effects on enzyme activities	24

6.4.	Factors of importance for fiber effects on enzyme activities	26
6.5.	Functional significance of in vitro effects of fiber on enzyme activities	27
6.6.	Summary	28
7.	FIBER EFFECTS ON NORMAL PANCREATIC FUNCTION	30
7.1.	Background	30
7.2.	Fiber effects on the feedback mechanism for pancreatic secretion	31
7.3.	Fiber effects on enzyme activities in the intestinal content	31
7.4.	Fiber effects on fat and protein digestion-absorption	32
7.5.	Fiber effects on starch digestion and postprandial glycaemia	33
7.6.	Summary	33
8.	FIBER EFFECTS IN PANCREATIC INSUFFICIENCY	34
8.1.	Background	34
8.2.	Fiber effects on starch digestion and postprandial glycaemia-insulinaemia	35
8.3.	Fiber effects on intraluminal enzyme activities and on fat absorption	36
8.4.	Summary	39
9.	ENZYME SUBSTITUTION IN PANCREATIC INSUFFICIENCY ...	40
9.1.	Background	40
9.2.	Effects of enzyme treatment on steatorrhea	40
9.3.	Effects of enzyme treatment on pain from chronic pancreatitis	40
9.4.	Summary	42
10.	POSSIBLE CLINICAL IMPLICATIONS	43
11.	GENERAL SUMMARY	45
	ACKNOWLEDGEMENTS	46
	REFERENCES	47

The primary objective of the present study was to contribute to a better understanding of the entero-pancreatic relationships and their importance for prevention and treatment of pancreatic diseases, and especially for dietary, pharmacological and surgical measures in chronic pancreatitis. Because of the analogies between dietary fiber and enzyme inhibitors in respect of effects on faecal fat and nitrogen excretion, and on glucose metabolism, an investigation was done to elucidate dietary fiber effects on pancreatic digestive enzyme activities - especially since an increasing amount of fiber is recommended in current food regimens. As duodenal juice is the natural medium of the digestive activities of the pancreatic enzymes, *in vitro* incubations were performed to study fiber effects on enzyme activities, both in buffer solutions and in human duodenal juice. Since fiber was found to inhibit pancreatic enzyme activities in the intestinal content, including trypsin activity, it was of interest to study effects on the digestion of nitrogen, fat and starch, as well as effects on pancreatic secretion and on carbohydrate metabolism. Studies of these effects were done in the rat. As enzyme inhibiting effects were likely to have a greater impact in cases of enzyme deficiency, studies were further performed concerning effects of dietary fiber on enzyme activities of the intestinal content, and on fat digestion in patients with pancreatic insufficiency. Experiments were also done with regard to fat and starch digestion in pancreatic duct-occluded rats. Rats operated on with ligation or tissue glue occlusion of the pancreatic ducts were studied in order to define this animal model with regard to intraluminal enzyme activities. As duct occlusion interferes with intraluminal trypsin activity, and like the disease of chronic pancreatitis primarily causes damage to the exocrine pancreas, and further as duct occlusion is proposed as treatment in chronic pancreatitis, it was of interest to study the endocrine versus the exocrine pancreatic function in duct-occluded rats. Because of the suppressive effects of intraluminal trypsin on pancreatic secretion, and because pain in chronic pancreatitis may be due to increased intraductal pressure, a study was also done of effects of enzyme treatment on pain in chronic pancreatitis.

Against the above mentioned background the present thesis was focused on the following main subjects: 1) Effects of duct occlusion on acinar and insular function, 2) *In vitro* effects of dietary fiber on pancreatic enzyme activity, 3) *In vivo* effects of dietary fiber on pancreatic function, 4) Effects of dietary fiber in pancreatic insufficiency, 5) Effects of enzyme substitution on pain in chronic pancreatitis.

2. GENERAL BACKGROUND

2.1. Pancreatic function

The name pancreas (from Greek pan, all, and kreas, flesh) does not give a very appropriate idea of the intricacy of the organ, or rather the two organs, whose function is closely interrelated with that of other organs, mainly the intestine. The gland is composed of an exocrine and an endocrine part. The pancreatic juice secretion assists the digestion of food in the intestine, and is regulated by neuro-hormonal mechanisms. It is stimulated especially by a meal. Visual, olfactory, gustatory, and possibly psychic, stimuli act on the exocrine pancreas via the vagal nerves, either directly or by gastrin release from the stomach. Gastric stimuli influence the secretion by similar pathways. Thereafter, the most important signals that stimulate secretion, come from the proximal intestine and reach the pancreas by hormonal mediation or by a vago-vagal reflex arch. Inhibitory control is exerted mainly from the distal ileum and from the colon by hormonal and nervous ways. The entero-acinar relationships will be described in more detail below (2.3.).

The internal secretion of the pancreas is regulated by neuro-hormonal mechanisms and direct regulation by food constituents, mainly glucose. Insulin secretion is thus stimulated by blood glucose, by hormones and by vagal influences. Hormones and the splanchnic nerves mediate inhibitory effects on insulin release (see below).

The exocrine pancreas also influences the function of the endocrine pancreas, and vice versa. Insulin and pancreatic polypeptide (PP) exert stimulating effects, and somatostatin and glucagon inhibiting effects on exocrine function. Oral trypsin inhibitor administration affects endocrine function.

2.2. Entero-acinar interactions

The pancreas acts on the gut by its exocrine secretion that increases the volume, the pH, and the enzyme activities of the intestinal content.

The gut acts on the exocrine pancreas in several ways. Soon after Pavlov (93) demonstrated a neural stimulation of pancreatic juice secretion, Bayliss and Starling in 1902 (11) introduced the concept of humoral stimulation of the secretion. They showed that the action of hydrochloric acid on the mucosa of the proximal duodenum via a bloodborne agent stimulated to pancreatic juice secretion. The involved hormone was named secretin. It is released by intraluminal acid, bile and fat, and causes the secretion of a juice that is copious and alkaline but poor in enzyme content. Another hormone, isolated in 1943 by Harper and Raper (45) from the small

intestinal mucosa, induces the flow of a juice that is rich in enzymes but scanty in volume. It is released by the action of intraluminal amino acids, fat and possibly trypsin inhibitor, and is named cholecystokinin-pankreozymin (CCK). Longterm treatment with CCK injections leads to an increase in weight and enzyme content of the pancreas. During the last years even other intestinal polypeptides (106) have been found to influence pancreatic secretion. A stimulating effect has been noted by vasoactive intestinal peptide (VIP), gastric inhibitory polypeptide (GIP), and chymodenin, and an inhibiting effect by somatostatin, and by pancreotone, which latter hormone (or candidate hormone) is released from the distal ileum and colon. Table I lists the currently recognized neuro-hormonal mediators of stimulating and inhibiting effects, initiated from the intestine, and acting on the exocrine pancreas. Gastrin is included in the list, as it is involved in a neuro-hormonal entero-pancreatic reflex. Even the products of pancreatic secretion themselves exert a regulating influence on the pancreas via entero-pancreatic pathways. The effects of changes in the intestinal activities of trypsin and other pancreatic enzymes will be discussed below.

Table I. Entero-pancreatic regulation.

<u>Regulating intraluminal factors</u>	<u>Mediators</u>	<u>Exocrine response</u>	<u>β-cell response</u>
	gastrin	+	?
	secretin	+	+
	CCK	+	+
acid	GIP	+	+
bile	VIP	+	+
fat	GLI	?	+
protein	substance P	+	?
carbohydrate	chymodenin	+	?
trypsin	somatostatin	-	-
	pancreotone	-	?
	vagus nerves	+	+
	splanchnic nerves	-	-

+ = stimulation. - = inhibition. CCK = cholecystokinin. GIP = gastric inhibitory polypeptide. VIP = vasoactive intestinal polypeptide. GLI = glucagon-like immunoreactivity.

2.3. Entero-insular interactions

Since Banting and Best in 1922 (10) isolated insulin, other polypeptides have been found that are produced by the islets, like glucagon, somatostatin, and pancreatic polypeptide (PP). Via hormones the islets have got effects on the intestine: glucagon reduces gut motility, and somatostatin decreases secretions into the intestine.

In Table I are listed proposed entero-insular influences. The concept of an "incretin" effect was introduced in 1930 by LaBarre and Still (74), who induced hypoglycaemia in dogs by intravenous injection of a crude secretin preparation. Regulatory effects on insulin release have been ascribed to several gastrointestinal hormones (20) such as secretin, CCK, gastrin, GIP, VIP, neurotensin, substance P and somatostatin.

2.4. Importance of the intestinal activities of pancreatic enzymes for exocrine and endocrine pancreatic function

Changes in activity levels of pancreatic enzymes in the content of the proximal intestine have been ascribed influences on both exocrine and endocrine pancreatic function. Infusion of trypsin into the duodenum suppresses the secretion of pancreatic juice (60), whereas trypsin inhibitor stimulates the secretion of trypsinogen, lipase and amylase (60). This mechanism of feedback regulation of pancreatic secretion, which is thought to be mediated by gastrointestinal hormones, has been demonstrated in the chicken (19), the rat (41, 60), the syrian golden hamster (4), and in the pig (57). It has also been shown in man - in a patient with an ampullary tumor (58). Evidence has else been brought for (62, 91) and against (50) the existence of this feedback mechanism in man. Long-term administration of trypsin inhibitor has got a trophic effect on the pancreas, and preferentially increases pancreatic trypsin content (8). It also improves glucose tolerance and increases glucose utilization in peripheral tissues in normal and in alloxan diabetic rats. This effect seems to be largely insulin-independent (56, 80). Intraduodenal infusion of amylase or lipase in the rat does not reduce pancreatic secretion (75). Feeding an α -amylase inhibitor has been found to reduce the amylase content of the rat pancreas (35).

From clinical point of view increases in intraluminal activities of pancreatic enzymes can be achieved by oral enzyme treatment (59). Decreased intraduodenal activities of pancreatic enzymes may occur in pancreatic disease like chronic pancreatitis and pancreatic carcinoma (59, 79). It can also result from measures like total or partial pancreatectomy, and from oral intake of enzyme inhibitors (52). Food components may also change intestinal enzyme activities (24).

2.5. Treatment in chronic pancreatitis

Treatment in chronic pancreatitis is directed mainly against three dominating problems: pain, digestive insufficiency, and diabetes. The conservative regimen includes alcohol abstinence, analgesics, enzyme substitution for maldigestion, and treatment for diabetes. Intractable pain has been treated with resection of the pancreas, and with ductal drainage procedures (7). The rationale for drainage operations is that pain may be caused by increased intraductal pressure. Recently, occlusion of the pancreatic ductal system with a tissue glue has been performed, in most cases together with resection, as treatment for pain (37). With an aim to cause the secretion to cease, the operation is hoped to bring the inflammatory process to a painless "burnt-out" stage.

In view of the relationships that may exist between intraluminal enzyme activities on one hand and exocrine and endocrine pancreatic function on the other, it may well be that a treatment, effective for one symptom of chronic pancreatitis, at the same time might have a positive or negative effect on another aspect of the disease. Treatment with enzyme preparations, which help to alleviate steatorrhea, may - via entero-acinar and entero-insular regulatory adjustments of pancreatic function - also have a positive influence on pain and a negative one on endocrine function. Resections, which may have effect on pain, obviously increase the risk of maldigestion and diabetes. Effects of ductal occlusion on exocrine and endocrine function are incompletely clarified. Although some reports on pain-relieving effects are promising, it is not known if the operation is good or bad for the islet function. The importance of dietary factors in chronic pancreatitis is largely unknown. However, an excessive fat intake provokes steatorrhea, and possibly pain (100).

3. ANIMALS, PATIENTS, AND MATERIAL

3.1. Rats

Male Wistar rats weighing 250 g were used for experiments with bile-pancreatic duct fistulae, rats weighing 200 g for ileostomy experiments, and those weighing 100-150 g for other experiments. When not stated otherwise the animals were kept on tap water and standard pellet diet.

3.2. Patients and healthy volunteers

Nineteen patients with the diagnosis of chronic pancreatitis took part in a study on the effects on pain by enzyme substitution. There were 8 women and 11 men with a mean age of 43 and 47 years, respectively. In 10 cases alcohol overconsumption was the cause of the disease. Seven had diabetes mellitus. Healthy volunteers, patients operated on with total pancreatectomy for malignant neoplasm, and patients with chronic pancreatitis participated in the Lundh test, and in the triolein breath test. Informed consent was obtained from all participants in the experiments.

3.3. Chemicals, drugs and digestive media

Highly purified crystalline amylase (type II-A from Bacillus subtilis), Sigma Ltd, St Louis, Mo., USA, was dissolved at 0.5 mg/ml in 0.05 M NaHCO_3 ; pH 8.4; amylase activity per ml: 230 U. Porcine pancreatic lipase, a gift from Charlotte Erlanson, University of Lund, was dissolved at 0.14 mg/ml in 0.05 M Tris, 150 mM NaCl, 1 mM CaCl_2 , 0.02 % NaN_3 ; pH 7; lipase activity per ml: 120 U. Porcine pancreatic phospholipase by the same donor was dissolved at 0.02 mg/ml in 0.5 M Tris, 150 mM NaCl; pH 6.5; phospholipase activity per ml: 10 U. Trypsin (Trypure[®]) from Novo industri A/S, Copenhagen, Denmark, was dissolved at 0.25 mg/ml in 0.05 M sodium phosphate; pH 6.5; trypsin activity per ml: 30 U. A granulated pancreatic enzyme extract (Pankreon[®]) was delivered by Kali-Chemie, Hannover, BRD.

Highly purified bovine lung trypsin inhibitor was obtained from Bayer A/G, Leverkusen, BRD.

Guar, pectin with 37.4 % methylic esterification (LM pectin) and pectin with 73.7 % methylic esterification (HM pectin) were gifts from the Copenhagen Pectin Factory Ltd, Copenhagen, Denmark. Wheat bran with a particle size of 0.25-1.5 mm was delivered by Kungsörnen AB, Stockholm, Sweden. Isphagula powder is a mucilago obtained from the hulls of a seed, *Plantago ovata*, and is commercially available, e.g. from AB Tika, Lund, Sweden (Lunelax[®]).

Cyanoacrylate tissue adhesive (Bucrylat[®]) and prolamine (Ethicon[®]) were obtained from Ethicon, Hamburg, BRD. Alloxan Monohydrate was

purchased from British Drug Houses Ltd., Poole, England. Soluble starch pro analysi according to Zulkowsky GR was obtained from Merck, Darmstadt, BRD. Polyethylene glycol (PEG) with a molecular weight of 10,000 was from KEBO AB, Malmö, Sweden.

[^{14}C] triolein (tri [$1\text{-}^{14}\text{C}$] oleoylglycerol) was delivered by the Radiochemical Center, Amersham, UK. ^3H was a gift from Mr Lennart Krabisch, University of Lund, Sweden.

Bile-pancreatic juice used for infusion was collected on ice from rats with bile-pancreatic duct fistulae and frozen at -20°C the day before the experiments.

Human intestinal juice used for in vitro incubation studies was collected on ice from healthy volunteers after a Lundh test meal (79). Enzyme activities of pooled duodenal juice were: amylase, 73 U/ml, lipase, 301 U/ml, phospholipase, 12 U/ml, and trypsin, 37 U/ml. The pH was 5.7. One trypsin unit is here defined as the enzyme activity hydrolyzing 1 μmol p-toluenesulphonyl-L-arginine methyl ester (TAME) per minute; one amylase unit is the activity liberating reducing groups corresponding to 1 μmol maltose per minute; one lipase unit and one phospholipase unit are, respectively, the enzyme activity releasing 1 μmol fatty acids per minute when acting on respective substrate.

4. METHODS AND EXPERIMENTAL DESIGN

4.1. Biochemical assays

Protein was determined according to Lowry et al (78), as modified by Eggstein and Creutz (29). Amylase was assayed according to the method of Dahlqvist (22), or with the use of the Phadebas[®] kit, obtained from AB Pharmacia, Uppsala, Sweden; lipase was determined according to the method of Erlanson and Borgström (30) using tributyrine as the substrate, phospholipase by the method of Ihse and Arnesjö (53), and trypsin by a modified version of Hummel's method (51). Glucose was determined by a glucose oxidase method (83), insulin by radioimmunoassay (48), and starch and glycogen by the method of Rerup and Lundquist (96). Fat was measured as described by van de Kamer et al (112), and nitrogen according to Kjeldahl's method.

4.2. In vitro experiments

Dietary fiber was incubated with shaking at 150 r.p.m. with the use of a Dubnoff metabolic shaker for 1 h at 37°C with buffer solutions with amylase, lipase, phospholipase and trypsin, respectively. The following fiber preparations were used: LM pectin, HM pectin, and wheat bran at 1.0 g/100 ml (g%) and guar at 0.5 g%. Concentrations of wheat bran refer to net fiber concentrations. After wheat bran incubation, centrifugation was done, and enzyme activities were determined in the supernatants. After incubation with pectins and guar, centrifugation was not done. Enzyme activities of the buffer solutions, measured after 1 h at 37°C, were used as controls.

Pooled human duodenal juice was incubated for 1 h at 37°C with pectin, HM pectin, guar, wheat bran at 0.5-1.5 g%, with ispaghula at concentrations of 0.15-1.5 g% and with PEG at 14-50 g%. Enzyme activities were measured both before centrifugation and in the supernatants after centrifugation.

Before centrifugation the pH of the specimens was measured as well as the apparent viscosity, which was determined at a shear-strain rate of 105 s^{-1} with the use of a Contraves Rheomat 30 viscometer (117). Enzyme activities were also measured after the pH of duodenal juice had been reduced to 5.0, 4.5, and 3.9 by the addition of HCl, but without fiber. Enzyme activities were further also assayed after incubation of duodenal juice with the same volume of saline with fiber, i.e. preswollen fiber, and further after incubation, where the samples of saline with fiber had been acidified and neutralized before the incubation, and lastly, when acidification and neutralization had been done after the incubation. Duodenal juice - fiber incubations were also performed with different ionic strength and with different incubation times. Trypsin adsorption to wheat bran was examined by trypsin activity assays of an incubate of duodenal juice with 1.5 g% wheat bran

before and after repeated washings with a 0.05 M sodium phosphate buffer, pH 6.5.

4.3. Experiments in the rat

4.3.1. Pancreatic duct occlusion

Pancreatic duct ligation was performed in the rat by ligation of the bile duct around 5 mm from the duodenum and near the hilum of the liver. The bile flow was deviated with a catheter hepatico-duodenostomy. In a separate experiment an attempt was made to place the bile duct ligature as close to the duodenum as possible and proximally as high as possible - "complete duct ligation".

Tissue glue occlusion was carried out with the use of 0.1 ml of cyanoacrylate glue and prolamine, respectively. After ligation of the bile duct near the hilum of the liver, a needle was introduced transduodenally into the distal part of the bile-pancreatic duct for glue injection, while a ligature was temporarily tied around the distal part of the bile duct (Fig 1). Control rats had a sham laparotomy. At 4 weeks, and between 4 and 5 months an intravenous load of glucose, 0.5 g/kg body weight, was given in the conscious rat, and serial blood samples drawn for glucose and insulin assays. In rats operated on in a second experiment with "a more complete ligation" intravenous loadings were also performed at 18 weeks with glucose and sucrose, respectively, at 2 g/kg. A week after the last glucose load the rats were sacrificed by cervical dislocation, their pancreas examined for wet weight and amylase, and the small intestinal content for weight, amylase, lipase, and trypsin activities.

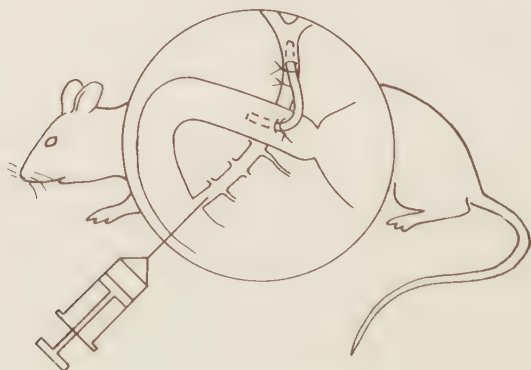


Fig 1. Occlusion of the pancreatic duct system with tissue glue. The bile is bypassed by means of a hepatico-duodenostomy.

4.3.2. Studies of secretagogue and trophic effects of dietary fiber and oral trypsin inhibitor

Via a laparotomy rats were provided with bile-pancreatic duct fistulae by the insertion of a polyethylene catheter into the bile-pancreatic duct through an incision in the duct close to the duodenum, and with duodenal fistulae by the insertion of another catheter into the duodenum 5 mm distal to the pylorus. Infusions were performed at 1.2 ml/h with bile-pancreatic juice containing 0.5 g% trypsin inhibitor, 1 or 1.5 g% LM pectin, or 5 g% wheat bran (2.5 g% fiber). Bile-pancreatic secretions were collected on ice at 30 min intervals for estimation of volume and amylase activity.

In another experiment one group of rats was given 2 ml of a solution with 5,000 KIU (kallikrein inhibitory units) per ml of trypsin inhibitor by daily gastric intubation. A control group got 2 ml water by the same route. These two groups were else fed the standard pellet diet. Four other groups of rats were fed different diets. One had a fiber deficient diet. To the same basal diet the other three groups had the additions of 5 % LM pectin, 5 % HM pectin and 20 % wheat bran, respectively. After 10 days feeding the rats of the six groups were sacrificed, and their pancreas glands were examined for wet weight and protein.

4.3.3. Fat and protein absorption experiments

After 12 hours' fasting rats were given 5 g of fiber deficient food or food with 5 % LM pectin. In both cases ³H-labeled triolein had been mixed into the fat of the diet in an amount giving 305,000 counts per 5 g food. After this feeding the rats were given standard feeding pellets. Faeces were collected for three days, and faecal isotope activity was measured after fat extraction with chloroform-methanol. The same experiment was done on rats operated on with duct occlusion with cyanoacrylate.

In another experiment rats were operated on with ileostomy. The distal ileum was divided about 5 cm from the caecum, and the proximal end of the ileum was stitched to the skin. The animals were kept in separate screen bottomed cages. After four days five groups of rats were fed 15 g/day of one of the following diets: 1) a fiber deficient diet, 2) one with 5 % HM pectin, 3) the same with 3 g Pankreon, 4) one with 20 % wheat bran, and 5) the same with 3 g Pankreon. After another three days they again had 15 g food, and ileostomy evacuates were collected two-hourly for 24 h for examination of wet weight, fat, nitrogen, and activities of amylase, lipase, and trypsin. After changing diets inbetween the groups, the rats were used for another study of the same kind.

4.3.4. Starch loading experiments

Three groups of rats were used: normal rats, rats operated on with

duct occlusion with cyanoacrylate glue, and rats made diabetic by intravenous injection of 43 mg per kg body weight of alloxan. By gastric intubation they were given 300 mg starch with and without 15 mg LM pectin. Serial sampling of blood was done for glucose and insulin determination. Starch and amylase in intestinal content were examined after 20-120 min. On normal rats blood glucose and insulin were also examined after a 300 mg gastric glucose load.

4.4. Human studies

4.4.1. Lundh test

After 12 hours' fasting intubation of the proximal intestine was performed on four patients operated on with total pancreatectomy and on five patients with chronic pancreatitis. A Lundh test meal with 5 ml Pankreon was given to the pancreatectomized patients with and without the addition of 5 g LM pectin, respectively, at two different occasions. Patients with chronic pancreatitis were given a test meal without Pankreon, but with or without 20 g wheat bran. In all tests intestinal juice was collected by siphonage, started immediately after the meal. Collections during the first hour were done in 10 minutes' fractions and during the second hour in 30 minutes' fractions. Enzyme activity determinations were done on a pool made up of 1 ml taken from each sample.

4.4.2. Triolein breath test

1.5-2 μ Ci [14 C]triolein was mixed with 100 ml Intralipid and given with 200 ml tap water after 12 hours' fasting to the participants of the study. After the meal CO_2 excreted with the breath was collected after 2, 4, 5 and 6 hours and examined for isotope activity. The sample with the highest activity was registered as peak $^{14}\text{CO}_2$ excretion, and the sum of activities of the different samples as cumulative excretion. Six healthy volunteers were examined as controls. Patients with total pancreatectomy were examined on three occasions: once after taking the fat meal containing ^{14}C -labeled triolein, a second time after the same meal to which 5 ml Pankreon was added, and a third time after the same meal with the addition of 5 ml Pankreon and 5 g LM pectin. Patients with chronic pancreatitis were examined twice: with and without the addition of 20 g wheat bran to the fat meal.

4.4.3. Effects of enzyme substitution on pain

In a double-blind study patients with chronic pancreatitis were examined during four weeks: the first week without treatment, the second with randomized treatment with 7.5 ml Pankreon granules 5 times a day, or with placebo made from heat-inactivated enzyme extract, the third week with no treatment, and the fourth week with verum- or placebo-treatment so that each patient had one week of Pankreon treatment and one with placebo. The patients kept daily

record of their symptoms, plotting the amount of pain on an analogue scale running from 0 to 100 mm. At weekly visits they were weighed, had liver and pancreas laboratory tests taken, and were interviewed by the examiner, who made an independent assessment of the mean amount of pain.

4.5. Calculations

The data were subjected to statistical analysis using the Student's t-test for paired and unpaired observations, and the X^2 test.

The experiments were approved by the local ethical committees.

5. EFFECTS OF PANCREATIC DUCT OCCLUSION ON PANCREATIC
FUNCTION
(I, II, VI, VIII)

5.1. Background

In 1922 Banting and Best (10), after ligating the pancreatic duct in the dog, found a subsequent exocrine atrophy with preserved endocrine elements. Pancreatic duct ligation has been studied in different species, and has been used therapeutically in man (92). Recently, occlusion of the pancreatic duct system with tissue glues has been suggested as treatment in chronic pancreatitis (37, 97). After prolamine duct occlusion in the dog Gebhardt and Stolte (39) found the development of an interstitial fibrosis and exocrine tissue atrophy, while the islets seemed unimpaired. However, it is still not very well known to what extent digestive function suffers, and in which way the islet function is affected by the operations of duct ligation and tissue glue occlusion.

Reduction of intestinal trypsin activity in the rat by trypsin inhibitor feeding causes an improved glucose tolerance (56). As treatment with CCK injections also improves the glucose tolerance (55), it has been suggested that CCK and/or other gastrointestinal hormones mediate the effects of trypsin inhibitor on the glucose metabolism. It could then be expected that duct occlusion, by reducing intraluminal trypsin activity, has got similar effects on the glucose tolerance.

5.2. Occlusion of the pancreatic duct system in the rat

5.2.1. Exocrine effects

Occlusion of the pancreatic ducts in the rat by ligation or by tissue glue occlusion caused a moderate exocrine pancreatic insufficiency as examined after about 20 weeks (I, II). The protein and the amylase activity per g pancreatic tissue, and the activities of trypsin, amylase and lipase in the intestinal content were moderately reduced. The reduction was more marked after tissue glue occlusion, and more marked with regard to the amylase activity as compared to the trypsin and lipase activity of the intestinal content. The trypsin activity of the intestinal content was reduced by one third after ligation and after prolamine occlusion, and by two thirds after acrylate occlusion. When examined two weeks after the operation with acrylate occlusion (VIII), intestinal amylase activity was only 1 % of that of normal rats as compared to 28 % at 21 weeks. Evidently, some restitution of digestive enzyme activity occurs with time after duct occlusion. At two weeks the weight of the intestinal content was greater than that of normal rats, which might reflect some malabsorption after duct-occlusion. A week after duct-occlusion with acrylate, faecal fat excretion was 60 times increased, and amounted to more than 10 % of the ingested fat as

measured after feeding with ^3H -labeled fat (VI).

The reason why the activities of digestive enzymes were not fully abolished in the intestinal content after ductal ligation or glue occlusion may be extrapancreatic enzyme sources (104), or the drainage of pancreatic ducts directly into the duodenum (49), or it may have to do with the technique of the operation. Pancreatic ducts might have been left to drain proximal to the ligature of the bile duct in the liver hilum, or distal to the distal ligature of the bile-pancreatic duct and in case of tissue glue occlusion distal to the temporarily applied ligature. That different surgical technique may cause a more or less marked exocrine dysfunction is shown by the different results after attempts to perform ductal ligation more or less completely. In the latter case only amylase activity was reduced in pancreatic tissue, while pancreatic protein remained unchanged, and in the intestinal content only lipase activity was reduced, while trypsin and amylase activities were unaffected.

It has previously been assumed that pancreatic duct occlusion resulted in a complete loss of pancreatic enzyme activity in the intestinal content. Our results that show remaining intraluminal enzyme activities, are supported by those of Arvanitakis and Folscroft (9), who four weeks after duct ligation in the rabbit, found unchanged faecal chymotrypsin activities. The finding in the present study of very low amylase activity in the intestine soon after duct ligation, later followed by increased activity, suggests that adaptive changes occur after some time. They might consist in a compensating increase of secretion from extrapancreatic enzyme sources, or in a gradually increasing secretion from pancreatic tissue outside duct-occluded areas, with remaining communication with the intestine. Intraluminal enzyme activities did, however, not return to normal in our experiments in the rat.

5.2.2. Endocrine effects

The endocrine effects on pancreatic function accompanying the exocrine atrophy in the present study were characterized by a preserved - or even improved - function of the β -cells. In pancreatic duct ligation experiments with a high degree of pancreatic insufficiency, the insulin response to an intravenous glucose load was much increased, and the insulin peak was registered at 7 minutes instead of 4 minutes. The glucose tolerance was improved. In rats operated on with a "less complete" duct-ligation, the insulin secreting capacity after I.V. glucose was slightly improved after 18 weeks, and the insulin peak was delayed. After four weeks the insulin release was unchanged. At both points of time the glucose tolerance was unaffected. In the latter group of rats the intestinal trypsin levels were not significantly lowered. In prolamine-occluded rats, in which the intestinal trypsin levels were reduced to a similar degree as in rats operated

with a "complete" duct ligation, glucose tolerance and insulin secretory capacity after I.V. glucose were increased both after four and 18 weeks, although the insulin peak was much less elevated than for rats with "complete" duct-ligation. In acrylate-occluded rats the glucose-induced insulin release was slightly impaired after four weeks, but improved at 18 weeks, when the insulin peak also was delayed. Possibly the islets at four weeks suffered an initial damage after acrylate-occlusion, which, however, did not cause any impaired glucose tolerance. In another type of experiment with oral starch loading an improved insulin release with a peak registered at 30 minutes instead of 15 minutes, and a reduced plasma glucose response were seen already two weeks after giving a starch load via an oro-gastric tube to acrylate-occluded rats. In histological sections at 18 weeks after tissue glue occlusion the number of islets seemed to be slightly fewer by visual impression, but of normal shape, whereas the exocrine parenchyma was atrophic.

The reason for the improved insulin secretory capacity seen in the present study, both after duct ligation and glue occlusion, is unexplained. In accordance with our results at four weeks after "incomplete ligation" Goberna et al (40) did not find any change in insulin release or in glucose tolerance 3-4 weeks after duct-ligation in the rat. During eight months after duct-ligation in the rat, fasting blood glucose levels were unchanged according to a study by Edström (28). In the dog, on the other hand, Ambromovage et al (1) noted reduced glucose tolerance after duct-ligation. Papachristou did not find any impaired islet function after duct-ligation in conjunction with pancreas resection in man (92). After acrylate duct occlusion in the dog, Little et al (76) found unchanged blood glucose levels and well preserved islets surrounded by fibrosis. The same authors did not observe any signs of impaired endocrine function after acrylate duct occlusion in six patients operated on for chronic pancreatitis (77).

5.3. Exocrine-endocrine relationships

A relationship between intraluminal trypsin activity and endocrine pancreatic function has recently been discussed by Melmed et al (86), and Ihse et al (61). An improved glucose tolerance and glucose utilization in peripheral tissues has been observed after long-term trypsin inhibitor administration, causing decreased intestinal trypsin activities (56, 80). These results were found both in normal and in alloxan diabetic rats (80). The observation of a stimulating effect of trypsin inhibitor on the islets is in accordance with the findings by Fujita et al (36) of an increased insulin content in the pancreas after oral trypsin inhibitor treatment in the normal rat, and with Bonnevie-Nielsen's reports of similar results from experiments in the mouse (13).

Cholecystokinin improves glucose tolerance in rats (55), and has

got trophic effects on the islets (36). Its endocrine effects are, however, not quite similar to those of trypsin inhibitor (55). It seems therefore probable that reduced intraluminal trypsin activity exerts a stimulating effect on the carbohydrate metabolism by the mediation of gastrointestinal hormones, though probably not only by cholecystokinin and not only by affecting islet function but also by a direct effect on peripheral tissues (80). With regard to the effect of duct occlusion an activation of the entero-insular axis would explain the observed effects on the endocrine pancreas. The delayed insulin peak seen in duct-occluded rats, especially after a gastric starch load, could have its cause in a mechanism, in which gastrointestinal hormones are involved. That the stimulating effects of duct-occlusion on the islets are of a sustained character is suggested by the findings of Andersson et al (2) of an improved glucose-stimulated insulin secretion *in vitro* from slices of pancreatic tissue from duct-ligated rats. These authors speculated that the observed effect might be due to increased proteolytic degradation of insulin in control tissue, or to improved islet nutrition in duct-occluded pancreas.

If intestinal trypsin activity constitutes one regulatory component of an "entero-insular axis", the present results seem to indicate that it is not the only factor of importance, since similar degrees of trypsin activity reduction are not always followed by similar effects on insulin release. If reduced intraluminal tryptic activity causes an improved glucose metabolism, it seems further that even moderate reductions in trypsin activity may be of importance. This should mean that moderate changes in trypsin activity, from pathological conditions or by nutritional factors, could have an indirect influence on carbohydrate metabolism. According to a report by Kalk et al (69) some patients with severe exocrine insufficiency from chronic pancreatitis had normal insulin responses to oral glucose. In nearly all patients in their study an insulin secreting capacity was retained. This could be due to stimulatory effects of gastrointestinal hormones.

Duct-occlusion in the rat may thus serve as an animal model for a moderate exocrine pancreatic insufficiency with preserved endocrine function. No good animal model has been devised for the study of chronic pancreatitis. With two years of alcohol treatment to rats, lesions very similar to those of alcohol pancreatitis in man can be seen (99). Experiments using such animals will be quite expensive. The operations with tissue glue duct occlusion in the rat provides a model, which is quickly available for studies on a subject with exocrine insufficiency, and which can be suitable for certain experiments that pertain to questions on chronic pancreatitis.

If duct-occlusion is used therapeutically in chronic pancreatitis, the effects on the exocrine pancreas of further reduced function may not be important, provided enzyme treatment can give a good substitution of the digestive enzyme activities in the intestine.

Any effects on the endocrine pancreatic function need not be unfavourable.

5.4. Summary

Pancreatic duct ligation or duct occlusion with tissue glues in the rat causes an exocrine pancreatic insufficiency, which - though it may be pronounced initially - is of a moderate degree. The influence of the procedure on the endocrine pancreatic function - although there may be a slight impairment initially - is one of preserved or even improved glucose tolerance and increased insulin secretory capacity. This influence on endocrine pancreatic function may at least partly be due to an effect of reduced intraluminal tryptic activity on gastrointestinal hormone(s).

6. EFFECTS OF DIETARY FIBER ON PANCREATIC ENZYME ACTIVITY IN VITRO
(III, IV)

6.1. Background

A high intake of dietary fiber is known to increase faecal fat and nitrogen losses (5, 12, 32, 47, 70, 73, 107, 108, 114). Whether these losses are due to maldigestion-malabsorption or to endogenous losses is not known. Furthermore, in recent years it has been seen that intake of certain fiber preparations improves glucose tolerance (64, 65, 67). This is an effect which has also been noted after oral trypsin inhibitor administration (56). These influences by fiber on digestion and on glucose metabolism motivated an interest in examining the in vitro effects of fiber on digestive enzyme activities.

Already in 1897 Brunton and Tunncliffe (17) performed in vitro digestions of brown and white meal, when they measured released sugars and residual nitrogen after incubation and filtration. They observed that the white meal was better digested than the brown by the saliva, liquor pepticus and liquor pancreaticus. The brown meal contained 1.6 % and the white meal 0.4 % crude fiber. During the last decade epidemiological studies by Burkitt (18) and Trowell (110) have inspired to an intensified research into the pathophysiological importance of dietary fiber, and a few in vitro digestive studies have been done. Without describing the details of their experiments, Westhuizen et al reported in 1972 (116) that starch in bread was much better digested by amylase in vitro than starch in cooked maize. The bread contained half the amount of fiber as compared to the maize meal. These two works suggest a detrimental effect by fiber on digestive enzyme activity. However, the two digestive processes that are compared in each case, are different in several respects and may therefore not be comparable. The substrate is different. The content of different dietary components and the particle size may be quite different.

In 1974 Arnal-Peyrot and Adrian (6) studied a digestive process with and without a fiber addition. They incubated soya protein with pepsin, papain and eripisin respectively, and maize starch with amylase. The presence of baobab gums and mucilages reduced the digestive effects, especially that of papain. In her thesis of 1976 (42) Greenberg described the incubation of different flour preparations with buffered solutions of enzymes. She had observed that wholemeal rye flour slowed down the amylase activity in vitro. Results of fiber effects on trypsin and pepsin activities were, however, inconclusive. Recently, Schneeman (101) demonstrated small decreases in trypsin and chymotrypsin activities in buffer solutions, and marked decreases in lipase activity, after incubation with different fiber preparations.

As the digestive processes may proceed differently in buffer solutions of pancreatic enzymes and in the duodenal juice, and as it is of greater interest to know about fiber effects on pancreatic enzymes in their physiological milieu, we studied these effects both in buffer solutions and in human duodenal juice.

6.2. Fiber preparations

Dietary fiber is a complex group of substances comprising plant polysaccharides and lignin, which are not hydrolyzed by the digestive enzymes of man. For the present study certain preparations have been chosen, as they have attracted interest for their effects on digestion and on carbohydrate metabolism, and as they - although in very different quantities - are commonly used in food. Pectin is a polymer, mainly built on galacturonic acid, and occurs with high methoxyl esterification (HM pectin) or low methoxyl esterification (LM pectin). Guar gum is a polymer of galactomannan. Wheat bran contains mainly cellulose and hemicellulose but also non-fiber components like starch and protein. Ispaghula consists mainly of a highly branched arabinoxylan. The fiber concentrations used in the incubation experiments described below are such that could occur in the duodenal content after high fiber intake. It is noted that different pectins vary considerably in physical properties as well as in effects on enzymes and, reportedly, also in effects on cholesterol metabolism (31).

6.3. Fiber effects on enzyme activities

At experiments, where pancreatic enzymes in buffer solutions were incubated for 1 h at 37°C with fiber, amylase and lipase activities were found to be markedly reduced by 1 g% LM pectin, and lipase and trypsin moderately reduced by 1 g% HM pectin. Phospholipase activity was slightly reduced by 1 g% guar gum, and trypsin activity was slightly reduced by 1 g% wheat bran.

In human duodenal juice (Fig 2) trypsin, lipase, phospholipase and amylase activities were much reduced by 1 g% LM pectin. The activities of the same enzymes, in particular lipase and phospholipase, were markedly reduced by 1.5 g% HM pectin. Lipase and amylase activities decreased by 50 % after incubation with 0.5 g% guar; and trypsin, lipase and amylase activities were all reduced by about 40 % by 1.5 g% wheat bran. Ispaghula, at 0.15 g%, only reduced the activity of lipase, which was decreased by 30 %.

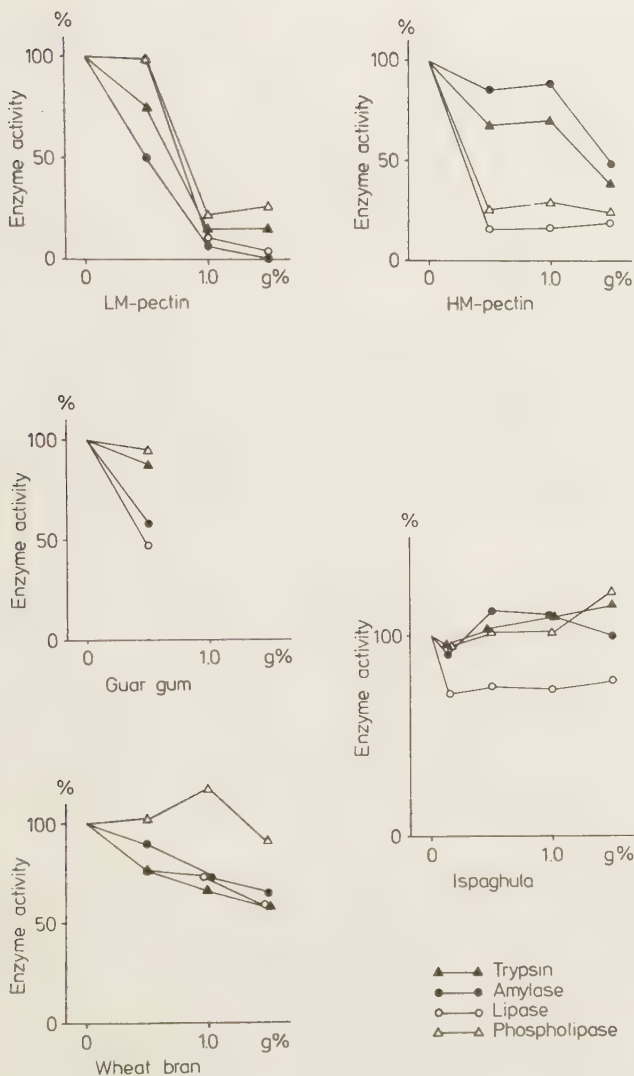


Fig 2. Changes in the activities of trypsin, amylase, lipase and phospholipase of human duodenal juice after incubation for 1 h with different fiber preparations. The enzyme activities are expressed as percentages of activities of duodenal juice without fiber. (From paper no. III)

6.4. Factors of importance for fiber effects on enzyme activities

Further incubation experiments showed an importance of viscosity, pH, and adsorption for the fiber effects on enzyme activities. HM pectin and guar gum considerably increased the viscosity of duodenal juice. When the viscosity of duodenal juice instead was increased with the use of polyethylene glycol, a reduction in enzyme activities could also be noticed, especially in the activity of lipase - i.e. the same enzyme activity on which HM pectin and guar showed particularly strong inhibitory effects. The importance of pH was demonstrated in an analogue way. LM pectin caused a reduction of the pH of the duodenal juice. If instead the duodenal juice was acidified with the use of hydrochloric acid, the activities of especially amylase and lipase were affected - i.e. the same enzyme activities that in particular were reduced by LM pectin. The effect of wheat bran on trypsin activity was shown to be, at least partly, due to an enzyme-adsorbing effect of bran. This adsorption could be reversed by buffer washings. On the other hand changes in ionic strength, and in incubation time, did not appear to have much importance for the effects of fiber on enzyme activities.

The importance of preswelling and pre-acidification of fiber - a treatment that mimics gastric passage - was also examined. Preswelling of fiber in saline did not change, or slightly augmented, the inhibiting effect of fiber on enzyme activity. An exception was the inhibiting effect of guar on amylase activity, which was impaired by preswelling. Exposure of fiber to an acid milieu prior to the incubation with duodenal juice was found to enhance the effect of some fiber preparations on enzymes. Changes in pH of the duodenal juice after incubation with fiber also had a marked influence on fiber effects, especially with regard to effects on amylase activity.

In contrast to our findings, Schneeman (101) did not find any inhibiting effect of pectin on enzyme activities in buffer solutions. She did, however, not study LM pectin, and the difference in results might else be explained by differences regarding the composition of the buffer solutions, the concentration of the fiber preparations and the centrifugation technique. She did not examine duodenal juice, in which medium we found greater fiber effects on enzyme activity. The reason for the difference in the present results concerning enzyme activities in buffer solutions and in duodenal juice may well be differences in respect of the buffering capacity and of the viscosity of the different media. In duodenal juice different unknown constituents may also be of importance. In 1981 Dunaif and Schneeman (26) reported on *in vitro* fiber effects on enzyme activities in still another medium, namely in diluted human pancreatic juice. Even here the authors did not find any inhibiting effects of citrus pectin, but substantial activity

losses of amylase, lipase, trypsin and chymotrypsin by Solka Floc (cellulose) and xylan (a hemicellulose), losses of trypsin and chymotrypsin activity by alfalfa, chymotrypsin activity losses by oat bran, and amylase and chymotrypsin losses by wheat bran. With wheat bran, we found slight activity reduction of trypsin in buffer solution, while we in duodenal juice found reductions in the activities not only of amylase but also of lipase and trypsin. Again, the differences between our results and those of Dunaif and Schneeman might have to do with different incubation conditions with regard to the media, the fiber concentrations and the centrifugation technique used.

In their study of the effects on enzyme activity of baobab gums and mucilages, Arnal-Peyrot and Adrian (6) noted that the inhibiting effect of fiber disappeared, if the fiber was first hydrolyzed. As the viscosity was lost by the hydrolyzing reaction, the finding is in agreement with ours regarding the importance of viscosity for the action of guar and HM pectin on enzyme activity. That citrus pectin and guar have got viscosity increasing effects has also been shown by Jenkins et al (67), who correlated this effect with that on mouth-to-caecum transit time and on glucose tolerance. Regarding our observation of the role of pH changes for the actions of LM pectin especially on amylase and lipase activities, it is noted that Gatfield and Stute (38) demonstrated a correlation between the detrimental effect on trypsin activity by carrageenan, pectin, and alginate, and an electrostatic interaction between the enzyme and the ionic polymers. In a short communication Schneeman reported (103) on the importance of pH for the inhibition effect on lipase activity by fiber. The activity decreased with increasing pH of the incubation mixture, in the range of pH 5.5-10 for Solka Floc, and 5.5-7.5 for xylan treated samples. pH levels lower than 5.5 might, however, also be of interest to study. In the present study after a test meal the duodenal aspirates of normal donors varied in pH from 5.25 to 6.65. Still larger variations are to be expected with different types of food as well as in disease. Especially pH-values below 5.0 were found to affect amylase and lipase activities of duodenal juice in the experiments of the present study. Adsorption to fiber of bile salts has been demonstrated by Eastwood et al (27). Our finding of trypsin adsorption to wheat bran has been confirmed by Besançon (personal communication), who found a 20-40% activity reduction of trypsin in buffer solution by adsorption to bran. The activity reduction was more marked when the pH was reduced, and also more marked when smaller particle size of bran was used. Ionic strength did not affect the bran influence on trypsin. He had also found inhibiting effects of pectin and xylan on activities of trypsin and chymotrypsin.

6.5. Functional significance of in vitro effects of fiber on enzyme activities

As preswelling and pre-acidification of fiber tended to enhance the

effect of some fiber preparations on enzymes, and as changes in pH of the duodenal juice had a marked influence on fiber effects, especially on amylase activity, it is not to be expected that fiber effects on enzyme activities will be lost by the gastric passage of fiber or by reductions in intraintestinal pH - but possibly by a rise in pH.

The capacity of digestive enzyme activities in the intestinal content is normally much greater than required. According to DiMagno (25) insufficient digestion of protein and fat only occurs when trypsin and lipase activities have decreased to under 10 % of normal levels. Regarding amylase activities Dahlqvist and Borgström (23) showed that the activities of the intestinal content are normally sufficient to digest starch completely in a few minutes.

Acute effects by fiber on digestion and absorption of nutrients in normal individuals are therefore unlikely to be of more than minor importance, as in vitro effects of fiber on enzyme activities were moderately pronounced. An exception was the quite pronounced effect on amylase and lipase activity by LM pectin at a concentration of 1 g% or more. These concentrations are not, however, likely to be reached in the intestinal content even after a high fiber intake.

If an individual's intraluminal activities of pancreatic enzymes are reduced, a further activity reduction by fiber might, however, be of importance, e.g. in cases of pancreatic insufficiency like chronic pancreatitis. If moderate reductions in intraluminal tryptic activity have got effects on the feedback mechanism regulating pancreatic function, then fiber effects on trypsin could perhaps be important even in other cases. At 0.5 g% HM pectin concentration, trypsin activities of duodenal juice were reduced by 35 %. The same concentration of wheat bran reduced trypsin activity by 25 %. These fiber concentrations might be attained in the duodenal content after a fiber rich meal, especially that of wheat bran. Preswelling and pre-acidification of the fiber, as would occur during the food passage through the stomach, would, according to the in vitro results, further enhance the inhibition of trypsin activity, and the effect on trypsin in the duodenum might then be greater. It was therefore of interest to study effects of fiber on digestive processes and - with regard to trypsin activity - on "indirect effects" on exocrine and endocrine pancreatic function via any influence on intraluminal tryptic activity, and to perform such studies both under conditions of an intact pancreatic function and under conditions of pancreatic insufficiency. Attention has been paid in the following to tryptic, lipolytic, and amylolytic functions. In vivo effects on phospholipase remain to be investigated.

6.6. Summary

Incubation of pancreatic enzymes in buffer solutions with dietary fiber showed some reduction of enzyme activities by fiber. In human

duodenal juice, however, enzyme activities were affected to a much greater extent by different types of dietary fiber. Amylase and lipase activities were reduced by pectins, guar and wheat bran, and trypsin by pectins and wheat bran. LM pectin had the strongest inhibitory effect on enzyme activities. Ispaghula reduced lipase activity moderately. A viscosity increasing effect was of importance for the influence of HM pectin and guar on enzyme activities, and a pH-reducing effect of LM pectin seemed to be involved in its influence on enzyme activity, whereas wheat bran was found to bind trypsin by adsorption. Preswelling and pre-acidification tended to enhance the fiber effects on enzyme activities.

7. FIBER EFFECTS ON NORMAL PANCREATIC FUNCTION (V, VI, VIII)

7.1 Background

As dietary fiber in vitro suppressed the activities of trypsin, lipase and amylase, maldigestion of protein, fat and starch could be expected to result from fiber intake. Suppression of trypsin activity could further result in effects similar to those of trypsin inhibitor treatment and thus interfere with the feedback regulation of pancreatic secretion (60), and with the regulation of endocrine pancreatic function (56).

It is well known that a certain degree of creatorrhea and steatorrhea can occur from a fiber-rich diet (5, 12, 32, 47, 70, 73, 107, 108, 114). The reasons for fiber-induced faecal nitrogen and fat losses are, however, not established.

McCance and Walsham recorded in 1947 (85) a reduced fat digestibility of wholemeal wheaten flour, and McCance and Glaser noted in 1948 (84) that the digestibility of fat and protein in oatmeal was reduced. Even Kay and Truswell (70), and Cummings (21) reported studies that suggested that increased faecal fat after fiber intake represents a change in the digestive and absorptive processes in the gut. Fiber-induced steatorrhea and creatorrhea have also been related to so-called endogenous losses, i.e. fat and nitrogen losses by mucosal desquamation, by secretion from the gastrointestinal tract, or by excretion of bacteria or of products resulting from the metabolism of intestinal bacteria. The influence of fiber on faecal fat excretion was studied by Walker (113) on Bantu school children, whose habitual diet was rich in fiber. When the children ate more fat but remained on the same (high) fiber intake, faecal fat excretion was not increased. If instead the fat content of their diet was unchanged, but the fiber intake increased by the addition of an orange, the faecal fat excretion was found to increase. The author interpreted these findings such that the steatorrhea was of endogenous origin.

It has been shown by Arnesjö et al (8) that oral trypsin inhibitor treatment to rats causes an increased weight and protein content of the pancreas gland. Furthermore, as shown by Ihse et al (60) intraduodenal administration of trypsin inhibitor stimulates the secretion of pancreatic juice. Long-term trypsin inhibitor treatment also improves glucose tolerance in rats (56).

In a follow-up of our abovementioned in vitro studies, the following experiments were performed in order to analyse in vivo effects on the intact pancreas: studies of 1) secretagogue and trophic effects of dietary fiber, 2) fiber effects on intestinal activities of pancreatic enzymes, 3) influence of fiber on fat and protein digestion-absorption, and 4) fiber effects on starch digestion, and on postprandial glycaemia and insulinaemia.

7.2. Fiber effects on the feedback mechanism for pancreatic secretion

Experiments were done on rats with duodenal and bile-pancreatic duct fistulae. Like trypsin inhibitor, when infused together with bile-pancreatic juice into the duodenum, so also LM pectin and wheat bran were found to stimulate the secretion of amylase from the bile-pancreatic duct. In another experiment, where rats were fed different diets for 10 days, wheat bran and HM pectin, like trypsin inhibitor, increased the protein content of the pancreatic gland. Wheat bran and trypsin inhibitor also increased pancreatic weight. These results show that dietary fiber may interfere with the previously mentioned feedback regulation of pancreatic secretion and, like trypsin inhibitor, exert secretagogue and trophic effects on the pancreas. The results are supported by those of Sheard and Schneeman (105), who found increased total activities of lipase, amylase, trypsin, and chymotrypsin in the pancreas of rats that had been fed for 10 days with a diet with added wheat bran. Also Fofana et al (34) reported that 2 weeks of wheat bran feeding to rats increased lipase and trypsinogen activities in the pancreas.

The effect of trypsin inhibitor administration on pancreatic secretion has been proposed to be mediated by gastro-intestinal hormones (52). Treatment with CCK injections was found to have trophic effects on the exocrine pancreas, according to a study by Ihse et al (55). A reliable radioimmunological method for CCK assay is not yet available (46). A support for the theory of CCK-mediated feedback stimulation was, however, provided by Brand and Morgan (15), who with the use of a bioassay method demonstrated a marked release of CCK-like activity from the proximal small intestine of the rat 20 minutes after intragastric trypsin inhibitor administration. Potent but brief stimulation of the pancreatic secretion followed the release of CCK-like activity, documented by a rise in intestinal amylase activity and a decrease in pancreatic amylase activity.

7.3. Fiber effects on enzyme activities in the intestinal content

On theoretical grounds different changes in the levels of enzyme activities in the intestinal content can be expected as a result of an inhibition of pancreatic enzyme activities by fiber. In the first place activities might be reduced from direct enzyme inhibition. Secondly, in an individual with intact pancreas and a feedback regulation of pancreatic secretion exerted by intraluminal trypsin activity levels, administration of a trypsin inhibiting factor would be expected to cause an increased secretion of pancreatic enzymes. In case of chronic trypsin inhibitor administration (8) a preferential increase in pancreatic trypsin content will be seen.

Thus, the effects of fiber intake with regard to enzyme activities in the intestinal content could then be a) reduced activities of pancreatic enzymes, b) increased enzyme activities, or c) increased activity of trypsin and reduced or unchanged activities of lipase and amylase. Enzyme activity levels may depend on the length of time of feeding the particular diet and on the time since the last meal was taken. The unpredictability of the results is demonstrated by the findings of the present study and of other studies. In ileostomized rats total lipase activity and amylase activity per g intestinal evacuate were increased after 3 days of feeding a diet to which HM pectin had been added. After the addition of wheat bran to the diet, total trypsin activity was increased, whereas amylase and lipase activities were unchanged. The response to 3 days of wheat bran feeding therefore corresponds to the effect of chronic trypsin inhibitor feeding. However, Sheard and Schneeman (105), after feeding rats wheat bran for 10 days, did not find any changes in intestinal activities of enzymes. In a study by Schneeman and Gallaher (102), in which rats were fed a diet with 20 % cellulose for 10 days, and killed 30 min after the last meal, both activities per g and total activities of trypsin, amylase and lipase of the intestinal content were reduced.

7.4. Fiber effects on fat and protein digestion-absorption

In experiments on ileostomized rats, 3 days' addition of 20 % wheat bran (10 % fiber) to the diet caused increases in the nitrogen and fat content of the ileostomy evacuate. The nitrogen contained in the wheat bran might possibly account for the creatorrhea, but the fat of the wheat bran could not explain the degree of steatorrhea. Feeding with 5 % HM pectin resulted in increased fat, but not nitrogen, losses. With regard to the results of pectin-feeding, they agree with those of a study by Sandberg et al (98) on ileostomy patients, whose ileostomy effluents contained more fat after the addition of 15 g pectin to the daily diet. With an amount of 16 g wheat bran added to the diet, the patients did not lose fat nor nitrogen from their ileostomies. This wheat bran intake was, however, considerably smaller, in proportion, than the one used in our experiments in the rat. Sandberg et al did not find any ileostomy starch losses from fiber intake. The results of our study and that of Sandberg et al imply that endogenous losses from the colon can not be the only cause of pectin induced steatorrhea. Maldigestion-malabsorption, or possibly endogenous losses from the small intestine, must be at least a contributing cause. Pectin induced creatorrhea, as well as bran induced creatorrhea and steatorrhea in man, would on the other hand result from endogenous colon losses. After high dose wheat bran feeding to rats the steatorrhea can, however, not be explained only by colon fat losses.

In another experiment, where normal rats were fed a meal containing ³H-labeled triolein, HM pectin increased the isotope activity of the feces. It follows from this study that maldigestion-malab-

sorption must be at least partly responsible for the steatorrhea caused by pectin. Whether lipase inhibition is a cause, or one of the causes, of the steatorrhea has not been established, but cannot be ruled out.

7.5. Fiber effects on starch digestion and postprandial glycaemia

Starch digestion was examined in normal rats with intact pancreatic function. The amount of starch remaining in the intestine 20 min after gastric administration of 300 mg soluble starch was increased from 1 to 6 % of the ingested amount by the addition of 15 g LM pectin to the starch gift. LM pectin reduced the plasma glucose response to a gastric starch load, but was also found to reduce the plasma glucose response to an equivalent glucose load to about the same degree. Pectin did not change the insulin response. It is concluded that delayed starch digestion is not likely fully to explain the fiber-induced glycaemia reduction after a starch load to normal rats.

7.6. Summary

Under experimental conditions in the rat, dietary fiber was shown to be able to interfere with the feedback regulation of pancreatic secretion, and to have secretagogue as well as trophic effects on the pancreas, i.e. effects which parallel those of a trypsin inhibitor. Changes were also seen after fiber feeding concerning activities of pancreatic enzymes in ileostomy evacuates. In high doses fiber was shown to increase ileal losses of fat and nitrogen, and slightly to delay the digestion of soluble starch.

8. FIBER EFFECTS IN PANCREATIC INSUFFICIENCY (VI, VII, VIII)

8.1. Background

Chronic pancreatitis is characterized by a progressive exocrine pancreatic insufficiency. Further, about every third patient will develop diabetes. In a material of patients with chronic pancreatitis examined by Ihse et al (54) intestinal activities of pancreatic enzymes were subnormal in about 80 % of the cases. The mean tryptic activity was about a third of normal in chronic pancreatitis patients, and the mean lipolytic activity was reduced to less than a fourth of the normal mean value. In severe exocrine insufficiency, i.e. when intraluminal lipase activities have decreased to below 10 % (25), steatorrhea will ensue. Creatorrhea may similarly appear if trypsin activities are reduced to less than 10%. Even starch malabsorption can be seen (81). Apart for the crucial role of alcohol, the importance of dietary factors for the course of chronic pancreatitis is understood only in a fragmentary way. Extreme malnutrition with regard to protein, and possibly even fat, has been associated with the development of chronic pancreatitis in underdeveloped countries (33, 109). On the other hand, an excessive fat intake increases steatorrhea, and has also been found to provoke pain and probably to hasten the progression of the disease (100). Even excessive protein intake has been considered harmful (100).

Dietary fiber has been proposed to be of beneficial value in diabetes (3, 64, 72). If it has got good or bad effects on the exocrine pancreas in chronic pancreatitis is not known. As fiber in vitro has exhibited detrimental effects on pancreatic enzyme activities (III), it could be expected that already reduced intraluminal enzyme activities in chronic pancreatitis might be influenced by fiber. Therefore, steatorrhea and creatorrhea in chronic pancreatitis may possibly be aggravated by fiber. A diet low in fiber has been claimed to predispose for diabetes mellitus (111, 115), and fiber has been thought to be of therapeutic and even prophylactic value with regard to diabetes. An improvement in postprandial glucose response has been referred to different possible effects by fiber. Jenkins et al (67) suggested that gel forming fiber delayed glucose absorption by delaying gastric emptying and diffusion in the intestinal content. Referring to the finding of reduced insulin/glucose ratio 45-90 minutes after a test meal, when guar and pectin had been added to the meal, Jenkins et al (65) speculated about fiber actions on the insulae via the release of gastro-intestinal hormones. Westhuizen et al (116) as well as Hansen and Schulz (44) demonstrated a reduced rate of starch digestion in the presence of fiber and suggested that this effect was related to an amylase inhibiting effect of fiber. Basing their theory on the concept of starch maldigestion from fiber, Wapnick et al (115) and Trowell (111) speculated in the possibility of a prophylactic importance of fiber intake with regard to

diabetes mellitus. In 1982 Jenkins et al (63) found evidence of a correlation between the effects of a food preparation on the rate of starch digestion in vitro, and the postprandial glycaemia seen after ingestion of the same food preparation. Reduced rate of starch digestion was related to the fiber content of the food.

Because of the trypsin inhibiting effects of fiber observed in vitro, fiber intake in pancreatic insufficiency might possibly bring about effects similar to those of oral trypsin inhibitor treatment. Trypsin inhibition effects mediated by gastrointestinal hormones could therefore be still another mechanism for fiber effects on glucose metabolism.

8.2. Fiber effects on starch digestion and postprandial glycaemia-insulinaemia

Starch loading experiments were done on rats operated on with occlusion of the pancreatic ducts with acrylate tissue glue, and on alloxan diabetic rats. In both groups of rats amylase activities of the intestinal content were much reduced. In duct-occluded rats they were less than 1% of that of normal rats, and in diabetic rats around 2 %.

In duct-occluded rats starch clearance from the intestine after a gastric load of 300 mg starch was impaired by 15 mg LM pectin, which caused an increase from 5 to 20 % of given amount of starch that remained undigested after 20 min. In the same rats the glucose response to a starch load was reduced by pectin. The reduction was considerably greater than that in normal rats. Even in diabetic rats pectin reduced the glucose response to a starch load considerably, and more than in normal rats. The insulin response after starch loading was not changed by pectin in any of the examined groups of animals. It can thus be seen that starch maldigestion is more apparent after pectin intake in animals with severe amylolytic insufficiency than in normal animals. Furthermore, postprandial glycaemia reduction by pectin was more marked in rats with amylolytic insufficiency than in normal rats. Starch maldigestion from amylase inhibition could possibly be a cause of this effect, although it hardly seemed to be of the same importance for the glycaemia reduction in normal rats (see above). Trypsin inhibition by fiber might also be involved, affecting the glucose response via changes in the pattern of gastro-intestinal hormone release. There was, however, no apparent change in the insulin response in neither normal rats nor in those with amylolytic insufficiency. In alloxan diabetic rats treated with an oral trypsin inhibitor for 3 and 5 weeks, Ihse et al found a slightly enhanced insulin secretory capacity as well as reduced basal blood glucose levels (61). In the present study (I, II), however, in rats with exocrine insufficiency - from duct ligation or occlusion - reduced intraluminal trypsin activity was seen together with improved glucose tolerance and increased insulin response after a glucose load. In a subject

with exocrine insufficiency it may therefore be that reduced intraluminal trypsin activity improves the glucose tolerance and stimulates the insulin release, whereas a normal subject gets an improved glucose tolerance without any apparent effect on the insulin release. As the duct-occluded rats did not react on fiber with an increased insulin response after oral starch load, the same mechanism may not be involved in case of fiber ingestion; or the same mechanism might be interfered with by the action of other gastrointestinal hormones that are affected by fiber. According to Morgan et al (89) serum GIP levels are reduced by fiber, and GIP stimulates the insulin release.

In duct-occluded rats the peak of the insulin response after an oral starch load was markedly enhanced, and observed at 30 minutes as compared to 15 minutes for normal rats. As previously discussed, the insulin release might be modified by a changed gastrointestinal hormonal influence on the β -cells. With pectin no change in the postprandial insulin response was seen, although the glycaemia was reduced. There are, however, reports about delayed or sustained insulin release, as well as reduced insulin levels, after high fiber intake. Jenkins et al (64, 65) observed that 10 g pectin and 16 g guar added to a test meal caused a sustained insulinaemia, lasting up to 90 minutes in healthy volunteers, and up to 120 minutes in diabetics. When studying the effect of meals with different fiber contents, Potter et al in 1981 (94) recorded a shift in the time of the insulin peak from 30 minutes after a meal containing less fiber, up to 60 minutes after a meal to which pinto beans were added. Furthermore, as shown by Jenkins et al (68), the addition of guar to a glucose meal resulted in an improved glucose tolerance when tested with a guar-free glucose meal given 4 h later. These observations of a sustained or delayed postprandial insulin release may be explainable by an effect modified by gastro-intestinal hormones.

8.3. Fiber effects on intraluminal enzyme activities and on fat absorption

The increase in fat losses seen after high-fiber diets in western countries has not been of that magnitude that it would have significant nutritional consequences. In patients with reduced intraluminal lipolytic capacity the case may be different. Fat absorption studies were therefore done on animals and patients with pancreatic insufficiency. In order to ascertain whether the steatorrhea after fiber intake is caused by malabsorption or not, experiments were done with isotope-labeled dietary fat.

In rats with acrylate-occluded pancreatic ducts, lipase activities were not reduced as compared to control rats 21 weeks postoperatively (II). As amylase activities were much more reduced one week after operation (VIII) than 21 weeks after, lipase activities may also have been reduced at one week after the operation. The ^3H -excretion with feces after a meal containing ^3H -labeled triolein in rats one week after duct-occlusion, corresponded to $12 \pm 1.3\%$ (mean $\pm$ SEM) of ingested triolein without, and $16 \pm 1.7\%$ with the addition of 5 % LM pectin to the meal. The difference was not significant - in contrast to the difference for normal rats (see above).

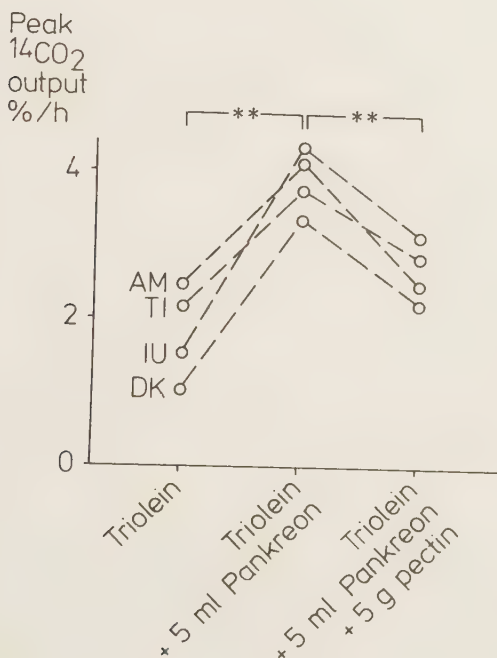


Fig 3. Peak $^{14}\text{CO}_2$ expiratory excretion in 4 patients previously operated on with total pancreatectomy, after ingestion of [^{14}C]triolein test meal, after [^{14}C]triolein test meal and 5 ml Pankreon, and after ingestion of [^{14}C]triolein test meal with 5 ml Pankreon and 5 g pectin. ** = p 0.01. (From paper no. VII)

In man studies with isotope-labeled fat were done both on patients with chronic pancreatitis - in whom lipase activities in the intestine were reduced - and in pancreatectomized patients, for whom a feedback mechanism of pancreatic secretion is out of question (VII). In the former group of patients, 20 g wheat bran, when added to a ^{14}C -labeled fat meal, reduced $^{14}\text{CO}_2$ expiratory excretion, indicating a reduced fat absorption. In pancreatectomized patients $^{14}\text{CO}_2$ excretion was about a third of normal, but increased with enzyme substitution to just above the lower level of normal. Five g LM pectin caused a decrease in $^{14}\text{CO}_2$ excretion to subnormal values (Fig. 3). In both categories of patients $^{14}\text{CO}_2$ excretion was reduced by fiber to levels that have been correlated with steatorrhea (90). Though confirmation could not be obtained from experiments on duct-occluded rats, the human studies gave evidence for fiber induced fat malabsorption. As fiber can also influence carbohydrate and bile acid metabolism (27, 71), such effects might interfere with the interpretation of the results of the triolein breath test. An influence of fiber on intraluminal pancreatic enzyme activities could, however, be demonstrated in the same patients by a Lundh test (79) in a way that agrees with the breath test results. Five g LM pectin added to the test meal, to which 5 ml Pankreon was added, reduced both trypsin, lipase and amylase activities in the pancreatectomized patients. The lipase activity was hereby reduced to levels where steatorrhea may occur. In patients with chronic pancreatitis 20 g wheat bran reduced intraduodenal amylase activity, whereas trypsin and lipase activities were not significantly reduced. If one patient, who had an extremely low lipase activity (6 U/ml), is excluded from the material, the lipase activity reduction was, however, significant. The different responses on enzyme activities in the two groups of patients could depend on different enzyme inhibiting effects of wheat bran and LM pectin - the effect of the latter being greater *in vitro* -, but could also have to do with a feedback response, modifying the effects in the chronic pancreatitis patients.

The results show that pectin and wheat bran can reduce intestinal activities of pancreatic enzymes in patients with pancreatic insufficiency, and also impair fat absorption. As lipase activities after fiber intake were under 10% of normal, and as $^{14}\text{CO}_2$ excretion after fiber intake at a triolein breath test was reduced to subnormal levels, there is strong evidence that dietary fiber in pancreatic insufficiency can cause steatorrhea by lipase inhibition. The finding of $^{14}\text{CO}_2$ excretion amounting to about a third of normal in pancreatectomized patients without enzyme treatment indicates the existence of extrapancreatic lipolytic activity of quite an important degree in man. This may originate e.g. from pharyngeal lipase or intestinal lipase (43, 104).

8.4. Summary

Experiments on pancreatic duct-occluded rats did not confirm fat malabsorption by fiber but showed starch maldigestion from pectin. In rats with amylolytic insufficiency pectin-induced reduction of postprandial hyperglycaemia after starch loading was greater than in normal rats. In patients with pancreatic insufficiency pectin and wheat bran reduced intestinal activities of pancreatic enzymes and reduced $^{14}\text{CO}_2$ excretion at a triolein breath test, indicating reduced fat absorption.

9. ENZYME SUBSTITUTION IN PANCREATIC INSUFFICIENCY (VII, IX)

9.1 Background

Enzyme treatment is used to treat malabsorption from exocrine pancreatic insufficiency and has been found effective in reducing steatorrhea, even if a normalization of the fat digestion seldom has been achieved (87). In a study by Ihse et al (59) pancreatic enzyme extract in granulated form was found to be more effective in increasing intestinal activities of pancreatic enzymes than extract in tablet form.

Although there has not been any controlled study to prove it, there have been reports that enzyme substitution in chronic pancreatitis has been seen to reduce pain. The cause of pain in chronic pancreatitis is not known. Operative drainage of the duct system has got a good effect on pain in 70-80 % (7). At operation for chronic pancreatitis with pain, Madsen and Winkler have registered increased pressure in the pancreatic ducts (82). As also high injection pressure at endoscopic retrograde pancreatography (ERP) provokes pain (Evander, personal communication), there is some evidence that pain, at least in some cases, is produced by high intraductal pressure. This theory has, however, been questioned by Bornman (14), who did not find morphological differences in the pancreatic ducts when performing ERP examinations on chronic pancreatitis patients with and without pain. In case a feedback mechanism exists in man, by which decreased intraluminal trypsin activity, probably hormonally mediated, stimulates pancreatic secretion, there is reason to expect effect on pain by oral enzyme treatment.

9.2. Effects of enzyme treatment on steatorrhea

When a granulated enzyme preparation was given with a Lundh test meal to pancreatectomized patients, trypsin and amylase activities in aspirates from the proximal jejunum were found to be normalized, and lipase activities were about 20 % of normal. The effects on intraluminal enzyme activities by a granulated enzyme preparation are in good agreement with the results by Ihse et al (59). As tested with the triolein breath test fat absorption was more or less normalized in the pancreatectomized patients by the same enzyme treatment.

9.3. Effects of enzyme treatment on pain from chronic pancreatitis

As a granulated enzyme preparation (Pankreon[®] granules) effectively increased intraluminal trypsin activity in patients with tryptic insufficiency (59), this preparation was used in patients with symptoms of pain from chronic pancreatitis.

In a double blind study Pankreon was found to improve pain in 15 out of 19 patients ($p < 0.05$) as compared to a placebo treatment, and to reduce the average pain for the patient population by 30 % both as recorded by the patients on analogue scales ($p < 0.01$), and as assessed by the interviewing examiner ($p < 0.05$). Even the frequency of pain attacks was reduced by Pankreon, which did not show effects on other symptoms, nor on laboratory tests on pancreatic and liver function. However, there were some patients in the study, in whom Pankreon had a very good effect on pain, while in some other patients the effect was weaker. It may therefore be that pain does not have the same cause in all cases, or that the enzyme treatment is not able to reduce an increased ductal pressure in all cases.

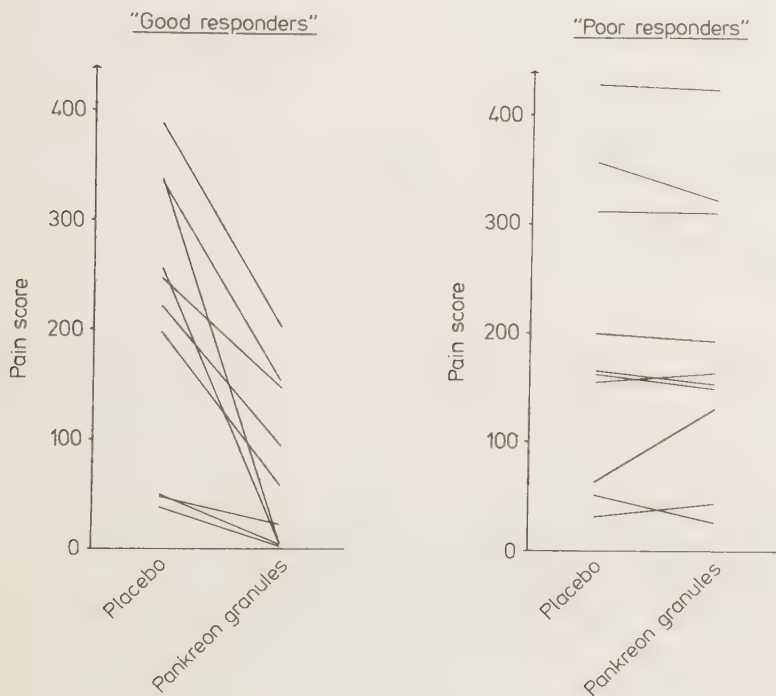


Fig 4. Pain scores as assessed by the patient for 10 patients with a "good response" and for 9 patients with a "poor response" to treatment with a granulated pancreatic enzyme preparation. (From paper no. IX)

The effect on pain by enzyme treatment provides another argument for the theory of a negative feedback mechanism for pancreatic secretion, governed by intraluminal trypsin activity. Evidence in the same direction is given by Jacobsen et al (62), who studied patients with chronic pancreatitis. They recorded decreased pancreatic chymotrypsin activity outputs after duodenal infusion of amino acids with trypsin as compared to infusion of only an amino acid solution. The effect was only seen in cases with a moderate pancreatic insufficiency. The results of our study and that of Jacobsen may suggest a feedback mechanism at least in patients with chronic pancreatitis. In 1977 Ihse et al (58) reported evidence for this mechanism in a patient with an ampullary tumor, who responded with stimulated pancreatic juice secretion to an intraduodenal infusion with trypsin inhibitor, whereas trypsin infusion caused a decreased secretion. Osnes and Hanssen (91), who performed secretion studies on man during endoscopic cannulation of the pancreatic duct, reported findings of a similar nature. On the other hand, Hotz et al (50) were unable to prove any regulatory effect of intraduodenal trypsin on pancreatic secretion, when they used a triple-tube technique on healthy subjects. As this technique uses insufflation balloons, other mechanisms than those initiated by intraluminal substances may, however, influence the results. As recently shown by Miyashita et al (88) upper jejunal distension with a balloon causes an inhibition of exocrine pancreatic secretion. The existence of a feedback mechanism in man is, however, still disputed, and more work is required before the question can be definitely settled.

The pain-relieving effect registered in the present study also gives reason to believe that pain in chronic pancreatitis, at least partly, or at least in some cases, is due to raised intraductal pressure. The findings of Bornman et al (14) of a similar morphology with regard to the incidence of ductal strictures in patients with pain and in those without pain does not exclude the possibility that the ductal pressure and the degree of morphological change are not correlated in individual cases. The present results give further reason to consider the possible influence of dietary factors on the disease development. A fatty meal, which is known to cause a strong CCK release, is reported to elicit pain (100), and might well do so by an effect on ductal pressure. Also dietary factors that affect intraluminal enzyme levels, like fiber (see above), might have an effect on ductal pressure and thereby on pain.

9.4. Summary

Enzyme substitution with a granulated extract was found to normalize fat absorption in patients operated on with total pancreatectomy. Enzyme substitution therapy to patients with chronic pancreatitis with pain was found to reduce pain intensity and pain frequency.

10. POSSIBLE CLINICAL IMPLICATIONS

From the present results of preserved or even improved β -cell function in the rat after longterm experiments of pancreatic duct occlusion with tissue glue, there appears to be reason to continue studies in man on the effects of this procedure as treatment for pain in chronic pancreatitis. In cases not suitable for surgical drainage procedures, resection is at times resorted to. This leads to an increased risk of causing a manifest diabetes, or of aggravating a diabetes. An alternative surgical method, that implies less risks for the endocrine pancreatic function, is desirable. Duct occlusion with a tissue glue like prolamine may be such an alternative. So far, prolamine occlusion operations have mainly been performed in connection with partial pancreatectomy (37). Even when partial pancreatectomy is performed for other indications than chronic pancreatitis, glue occlusion may be used in order to lessen operative risks by lessening the incidence of leakage from pancreatico-intestinal anastomoses. For the same purpose it may be used in pancreatic transplantation.

Although dietary fiber seems to have inhibiting effects on pancreatic enzyme activities in vitro, malabsorption is not likely to occur to a physiologically important extent in healthy people who eat the amounts of fiber that are used in Western countries. On the other hand, there may well be positive effects with regard to glucose metabolism. Fiber may be of prophylactic importance in cases of latent diabetes mellitus, and factors that are slowing the digestion and absorption of carbohydrates are of advantage in cases of obesity, diabetes and certain hyperlipidaemias (66).

Especially in patients with pancreatic insufficiency fiber might cause steatorrhea. As $^{14}\text{CO}_2$ excretion was decreased to subnormal levels by pectin in Pankreon-treated totally pancreatectomized patients, steatorrhea might be caused by fiber in exocrine pancreatic insufficiency, even in spite of enzyme substitution.

Pancreatic atrophy and even chronic pancreatitis has been noted in malnourished children (100, 109). The diet of malnourished children in developing countries is often of a high fiber content. In such cases fiber possibly increases a digestive insufficiency and could be thought to contribute to the malnutrition by impairing the digestion of the nutrients consumed.

Enzyme substitution is recommended as treatment for pain in chronic pancreatitis, even in the absence of steatorrhea. It may be questioned whether pain in chronic pancreatitis may be provoked by fiber, as enzyme treatment can reduce pain, and as fiber may inhibit enzyme activity. One may further speculate on whether possible trophic effects of fiber on the pancreas may have any influence on the course of chronic pancreatitis. It could also be questioned whether a trophic effect of fiber on the pancreas gland

may be of any importance with regard to carcinogenesis. There are, however, no epidemiological reports on any increased incidence of pancreatic carcinoma in African countries with a 4-5 times higher intake of fiber as compared to Europe.

The inhibition of phospholipase activity may reduce lecithin absorption, and thereby possibly also affect the cholesterol metabolism. As phospholipase activity is markedly inhibited by 0.5 g% HM pectin, and as highly methoxylated pectins have been found to reduce LDL cholesterol in plasma (31), there might possibly be a relation between the phospholipase inhibiting effect of pectin and its effects on plasma cholesterol.

11. GENERAL SUMMARY

Effects on exocrine and endocrine pancreatic function were studied after pancreatic duct occlusion in the rat. Studies were also done of dietary fiber effects on pancreatic enzyme activities in vitro, and on fiber effects in vivo on pancreatic function in the case of an intact pancreas, as well as in pancreatic insufficiency, in the rat and in man. In chronic pancreatitis, finally, enzyme substitution therapy was examined in respect of its effect on pain. From the results the following conclusions were drawn:

1. Pancreatic duct occlusion in the rat, effectuated by ligation or by tissue glue occlusion, caused an exocrine pancreatic insufficiency, which after four-five months was only of a moderate severity.
2. Four-five months after pancreatic duct occlusion in the rat the β -cell function was not impaired but rather improved. The glucose induced insulin release was increased.
3. Dietary fiber was found to exert inhibitory effects on pancreatic enzyme activities in vitro. These effects were more pronounced in duodenal juice than in buffer solutions.
4. In ileostomized rats dietary fiber changed ileal outputs of pancreatic enzyme activities and increased ileal losses of fat. The fiber-induced steatorrhea was, at least partly, due to maldigestion-malabsorption.
5. In the rat dietary fiber exerted secretagogue and trophic effects on the exocrine pancreas.
6. In patients with pancreatic insufficiency dietary fiber reduced intestinal activities of pancreatic enzymes, and reduced fat absorption.
7. In rats pectin delayed starch digestion and reduced postprandial glycaemia. Both effects were more pronounced in animals with amylolytic insufficiency.
8. Totally pancreatectomized patients did absorb some dietary fat even without pancreatic enzyme treatment. Treatment with a granulated enzyme preparation improved intestinal activities of pancreatic enzymes and normalized fat absorption.
9. Pancreatic enzyme substitution reduced pain intensity and pain frequency in chronic pancreatitis.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to:

Associate Professor Ingemar Ihse for initiating this study, and for teaching and guiding me throughout the work.

Associate Professor Ingmar Lundquist for invaluable help and constructive discussions.

Professor Stig Bengmark, my chief, for encouragement and support.

Professor Bengt Borgström for valuable criticism and advice.

Professors Nils-Georg Asp and Claus Rerup, Associate Professors Björn Åkesson and Bo Åhrén, Dr Sigmund Dawiskiba, and Dr Per Lilja for stimulating collaboration and discussions.

All members of the Pancreatic Team at the Department of Surgery, Lund.

Mrs Birgit Persson, Mrs Elisabeth Pollack, Miss Elisabeth Winberg, Mrs Lena Kvist, and Mr Peter Okmark for excellent technical assistance.

Miss Eva Edestad, Mrs Monica Lundin-Hansson, Miss Monica Keidser, and Miss Agneta Berglund for skilful secretarial and illustrative assistance.

Mr Pehr Johnson for illustrations and printing.

All patients and volunteers who participated in the study.

Pharmacia AB, and the Copenhagen Pectin Factory Ltd for generous provision of drugs and chemicals.

The investigation was supported financially by the Swedish Medical Research Council (projects no. 14X-4286, 14P-4289, 17X-3012 and 03X-3968), the Albert Pahlsson Foundation, the Medical Faculty, University of Lund, Sweden, Meda AB, and Tricum AB.

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PRINTING
ILLUSTRATION DEPARTMENT
DEPARTMENT OF SURGERY
UNIVERSITY OF LUND
1982.